

Interlaboratory Study of the
Cold Vapor Technique for
Total Mercury in Water

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Interlaboratory Study of the Cold Vapor Technique for Total Mercury in Water

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KEY WORDS: water quality, mercury (metal), environmental testing, statistical analysis, water pollution

The U.S. Environmental Protection Agency (EPA) gathers water quality data to determine compliance with water quality standards, to provide information for planning of water resources development, to determine the effectiveness of pollution abatement procedures, and to assist in research activities. In a large measure, the success of the pollution control program rests upon the reliability of the information provided by the data collection activities.

To ensure the reliability of physical, chemical, biological, and microbiological data, the Methods Development and Quality Assurance Research

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Laboratory (MDQARL), formerly the Analytical Quality Control Laboratory, was established in Cincinnati, Ohio, as part of the National Environmental Research Center (NERC-Cincinnati). The quality assurance program of this laboratory was designed to assure the reliability, and when necessary, the legal defensibility of all water quality information collected by EPA.

In MDQARL, the Quality Assurance and Laboratory Evaluation Branch is responsible for conducting all interlaboratory method studies for water and waste-water analyses in EPA.

Before this study, the chemists in EPA proposed a method of analysis for total mercury (organic and inorganic) in water.² The method includes an acid-permanganate persulfate digestion at 95°C for 2 h followed by reduction and measurement by the cold vapor technique. The same basic method was being considered also by Subcommittee D19.05 of Committee D-19 on Water, American Society for Testing and Materials (ASTM).

Because chemists in EPA also play an important roll in the task groups of ASTM Committee D-19, it was logical to propose a joint ASTM-EPA study for total mercury in water. This report describes the joint study that was conducted and its conclusions which include a statement of precision and accuracy for the ASTM-EPA method.

Experimental

Test Design

Youden's nonreplicate technique of x and y samples³ was used in a test design as follows:

1. Each sample was prepared as a stable concentrate in a sealed glass ampul.
2. Using Youden's x - y sample technique, similar yet different sample concentrates were prepared in pairs.
3. When an aliquot of a concentrate was diluted to volume, mercury was present at levels found in natural waters.
4. Four levels of mercury were tested to cover the normal levels found in surface waters.
5. Each of the x - y sample pair was added independently to a distilled water and natural water of choice and the samples were analyzed. The natural waters were analyzed with and without increments and the added levels determined by difference. Recoveries from distilled and natural waters were compared. Precision, accuracy, bias, and interferences were determined.

²Kopp, J. F., Longbottom, M. C., and Lobring, L. B., *Journal of the American Water Works Association*, Vol. 64, No. 1, 1972, pp. 20-25.

³Youden, W. J., *Statistical Techniques for Collaborative Tests*, Association of Official Analytical Chemists, Inc., Washington, D.C., 1967.

Details of the Study

Eight water sample concentrates were prepared for this study by dissolving weighed amounts of reagent-grade chemicals in ultrapure water (distilled water polished by passage through a Millipore 4 cartridge Super Q⁴ system) to produce accurately-known concentrations of organic and inorganic mercury. All eight samples contained the same ratio of inorganic to organic mercury, 40:60, as mercuric chloride and methyl mercury chloride, respectively.

The distilled water—natural water spike technique was used in this study. Each analyst was instructed to dilute a separate 5.0-ml aliquot of each concentrate to 1 liter with distilled water and a river, lake, or estuarine water of their choice. For each sample, a result was obtained for: (1) distilled water plus increment, (2) the natural water, and (3) the natural water plus increment. Recovery of the increment from natural water was determined by difference.

The concentrates were preserved with 0.15 percent redistilled nitric acid, and were checked for stability by repeated analyses over a period of three months. These analyses established that the solutions were stable and verified the concentration of each constituent. Further confirmation of the true values was obtained from determination by an independent referee laboratory. The true values are shown in Table 1. When diluted to volume according to the instructions, the samples contained the concentrations of total mercury in $\mu\text{g/liter}$.

Since dilution of the concentrates renders the acid ineffective as a

TABLE 1—True values for total mercury. ^a

Ampul	Mercury Concentration $\mu\text{g/liter}$	Ratio Inorganic/Organic Mercury
1	0.21	40/60
2	0.27	40/60
3	0.51	40/60
4	0.60	40/60
5	3.4	40/60
6	4.1	40/60
7	8.8	40/60
8	9.6	40/60

^aThe concentrations given are the actual levels calculated and added. These are not based on analyses, the latter being used only for verification.

⁴Mention of trade names or commercial products does not constitute endorsement by the EPA or ASTM.

preservative, the analyst was instructed to add 1.5 ml of redistilled nitric acid/liter during sample preparation.

Study Logistics and Methodology

The mercury study was announced in an invitational memorandum sent to each EPA regional Analytical Quality Control (AQC) Coordinator (regional representative for quality control) in September 1972. Further, the study was announced in EPA's AQC Newsletter which is distributed to about 7000 laboratories and technical offices of governmental and private agencies throughout the United States and Canada.

Seventy-six laboratories from EPA, Canada, other federal, state, and local agencies, universities, and the private sector took part. After a cut-off date of October 23 for responses, samples were packed and shipped on 30 Oct. 1972. Each laboratory was sent one set of eight sample concentrates, instructions for sample preparation, a copy of the full method writeup, and duplicate report sheets. The final date for reporting data was 20 Dec. 1972. All data returned to MDQARL by that date are included in this report.

The method developed by ASTM and EPA and evaluated in this study includes an acid-permanganate-persulfate oxidation procedure and heat, before reduction, to ensure conversion of all organic mercury compounds to ionic form before measurement. Kopp et al² described the method. In the present study, mercury was measured by atomic absorption spectrophotometers, ultraviolet/visible spectrophotometers, and meters such as the Coleman MAS-50 specifically designed for measurement of mercury.

Results

Screening for Nonuseable Data

Because the mercury study was done at low to fractional microgram/liter levels, some of the data received were orders of magnitude away from the true values. It was necessary to screen out these completely-extraneous values before beginning any data evaluations. If this was not done first, the standard deviation values calculated from these data would be excessively large and would misleadingly indicate large deviations for the method. Further, the rejection of outliers using the *t* test, which includes the standard deviation in its calculation, would be unable to identify outliers because of the overwhelming effect of the excessively large standard deviation values in the denominator of the *t* test.

With the need to reject the meaningless values before any data evaluation we chose to screen out values that exceeded four standard deviations. With normal distribution, this mechanism yields only six

chances out of 100 000 that a valid data point is being rejected. This probability is sufficiently small to allow use of this screening device before full data treatment.

Rejection of Outliers

To determine the accuracy of the analytical method, it was necessary to remove those extreme values that had only a small chance of validity and that would significantly change the reported precision and accuracy values. These values were probably caused by gross instrumental, chemical, or human error and were rejected by applying the two-tail *t* test to all values at a 99 percent probability level; that is, with a 99 to 1 assurance that the data rejected were invalid and should be rejected.

A greater spread of data round the true value causes rejection of fewer outliers because a larger standard deviation in the denominator of the *t* test reduces the calculated *t* value and fewer extreme values are rejected as outliers. With better accuracy and precision, the *t* test is more powerful and more outliers are rejected. However, the data rejected in either case are true outliers and laboratories reporting such values should carefully review procedures for the cause of inaccuracy.

Statistical Summaries

Statistical summaries are given in Tables 2 thru 5. Each sample is discussed in turn, with the data displayed by type of water sample and method of analysis. A statistical evaluation is provided for each concentration with each set of sample-test conditions by type of water sample.

All statistical parameters: mean, range, accuracy, standard deviation, and relative deviation are based on retained data, that is, the data remaining after rejection of outliers.

TABLE 2—Statistical summary of recovery of total mercury from distilled and natural waters.

Statistic	Sample 1		Sample 2	
	Distilled Water	Natural Water by Difference	Distilled Water	Natural Water by Difference
True value, $\mu\text{g/liter}$	0.21	0.21	0.27	0.27
Mean recovery, $\mu\text{g/liter}$	0.418	0.349	0.450	0.414
Accuracy as % relative error (bias)	98.9	66.4	66.5	53.3
Standard deviation, $\mu\text{g/liter}$	0.279	0.276	0.325	0.279
Relative deviation, %	66.9	78.9	72.2	67.5
Range, $\mu\text{g/liter}$	1.60	1.27	1.80	1.20

TABLE 3—Statistical summary of recovery of total mercury from distilled and natural waters.

Statistic	Sample 3		Sample 4	
	Distilled Water	Natural Water by Difference	Distilled Water	Natural Water by Difference
True value, $\mu\text{g/liter}$	0.51	0.51	0.60	0.60
Mean recovery, $\mu\text{g/liter}$	0.653	0.674	0.744	0.709
Accuracy as %				
relative error (bias)	28.1	32.1	24.0	18.1
Standard deviation, $\mu\text{g/liter}$	0.376	0.541	0.466	0.390
Relative deviation, %	57.5	80.3	62.6	55.0
Range, $\mu\text{g/liter}$	2.26	3.99	2.97	2.30

TABLE 4—Statistical summary of recovery of total mercury from distilled and natural waters.

Statistic	Sample 5		Sample 6	
	Distilled Water	Natural Water by Difference	Distilled Water	Natural Water by Difference
True Value, $\mu\text{g/liter}$	3.4	3.4	4.1	4.1
Mean recovery, $\mu\text{g/liter}$	3.40	3.41	4.26	3.81
Accuracy as %				
relative error (bias)	0.03	0.34	3.85	-7.11
Standard deviation, $\mu\text{g/liter}$	1.29	1.49	1.42	1.12
Relative deviation, %	37.9	43.7	33.2	29.3
Range, $\mu\text{g/liter}$	11.1	12.4	10.4	6.3

TABLE 5—Statistical summary of recovery of total mercury from distilled and natural waters.

Statistic	Sample 7		Sample 8	
	Distilled Water	Natural Water by Difference	Distilled Water	Natural Water by Difference
True value, $\mu\text{g/liter}$	8.8	8.8	9.6	9.6
Mean recovery, $\mu\text{g/liter}$	8.48	8.77	9.38	9.10
Accuracy as %				
relative error (bias)	-3.7	-0.4	-2.3	-5.2
Standard deviation, $\mu\text{g/liter}$	2.60	3.69	3.24	3.57
Relative deviation, %	30.7	42.1	34.5	39.2
Range, $\mu\text{g/liter}$	19.4	27.5	24.4	25.8

Two-Sample Charts

To effectively describe the recovery values obtained for each parameter, a series of two-sample charts, Figs. 1 to 8, were prepared as suggested by Youden.³ Results were plotted by parameter and by water sample type. Two results for each set of similar samples (1 and 2), (3 and 4), (5 and 6), and (7 and 8) were the coordinates on the x and y axes that determined a single point for each analyst. The distribution of points (analysts) characterized the method-results for that sample. However, the plotting of the statistically acceptable data for each sample pair resulted in severe crowding of data points $\leq$ to the true values, because of the large number of values greater than the true values. To expand the presentation of the data $\leq$ to the true value, plot scales were selected to place the true value at least one-third of the distance from the origin. This general rule proved acceptable and was used on all data plots. With its use, data points were distributed evenly over each plot to show a truly representative picture of method performance. Extreme values which characterize a small segment of the data were represented as $>$ values in the extreme upper right quadrant.

If random error was primarily responsible for the deviation of results from the true values, then the data from the sample pair would be equally distributed among the four quadrants $(++)$ $(-+)$ $(--)$ $(+-)$. How-

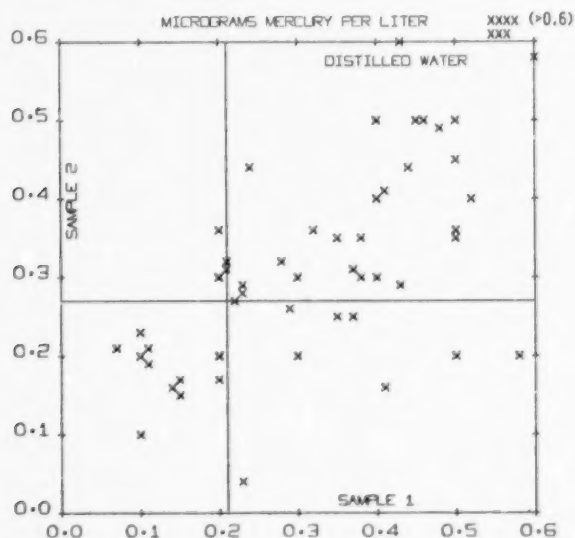


FIG. 1—Two-sample chart for total mercury, Samples 1 and 2.

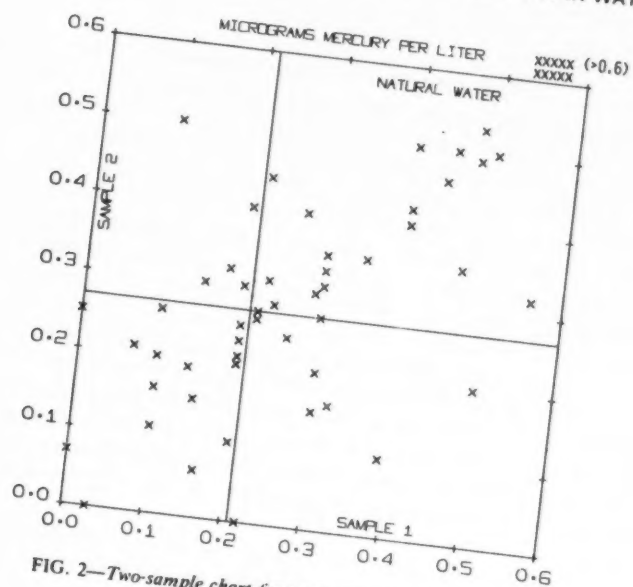


FIG. 2—Two-sample chart for total mercury, Samples 1 and 2.

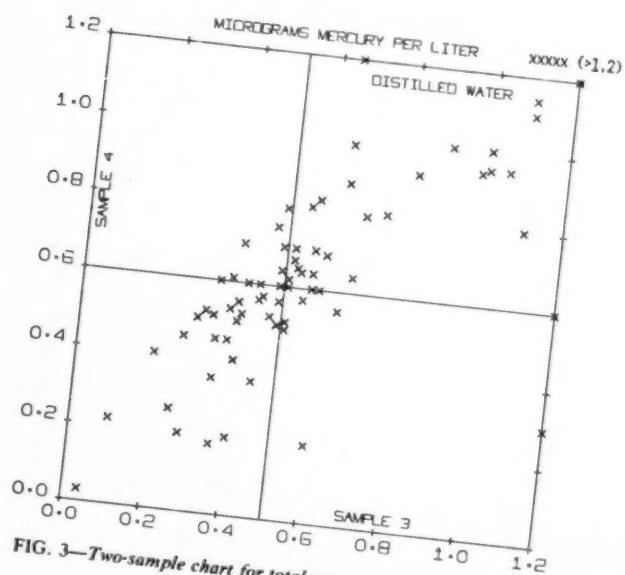


FIG. 3—Two-sample chart for total mercury, Samples 3 and 4.

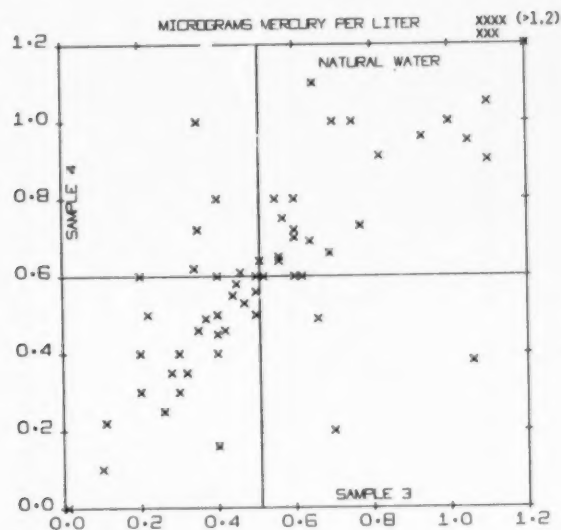


FIG. 4—Two-sample chart for total mercury, Samples 3 and 4.

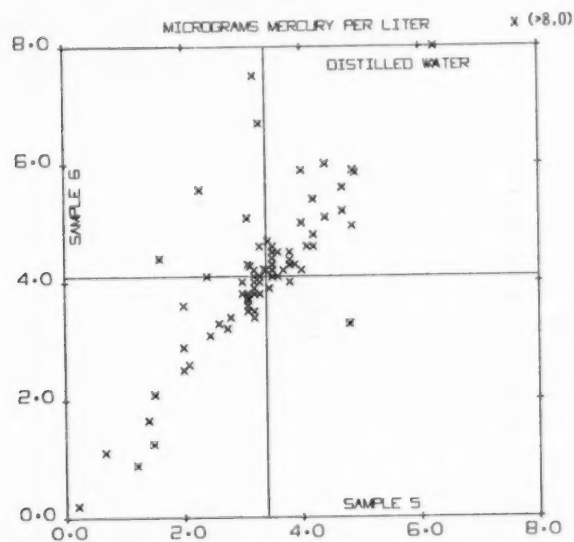


FIG. 5—Two-sample chart for total mercury, Samples 5 and 6.

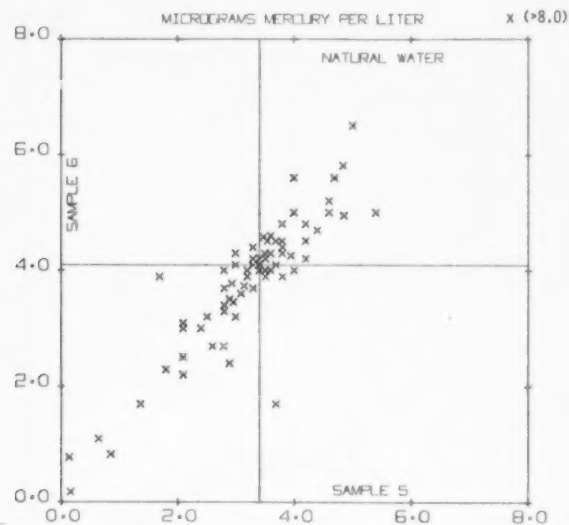


FIG. 6—Two-sample chart for total mercury, Samples 5 and 6.

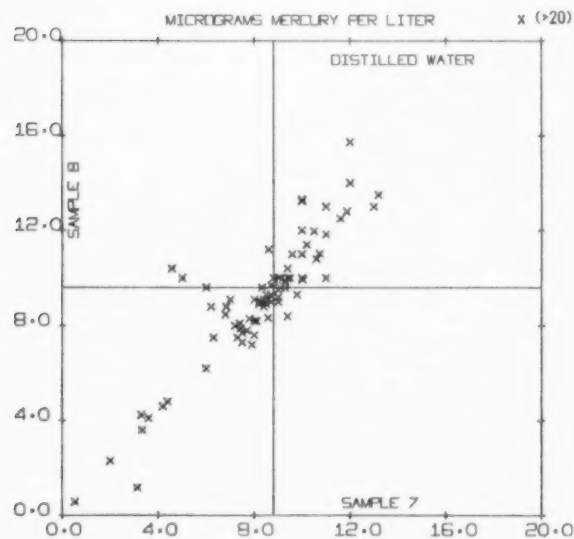


FIG. 7—Two-sample chart for total mercury, Samples 7 and 8.

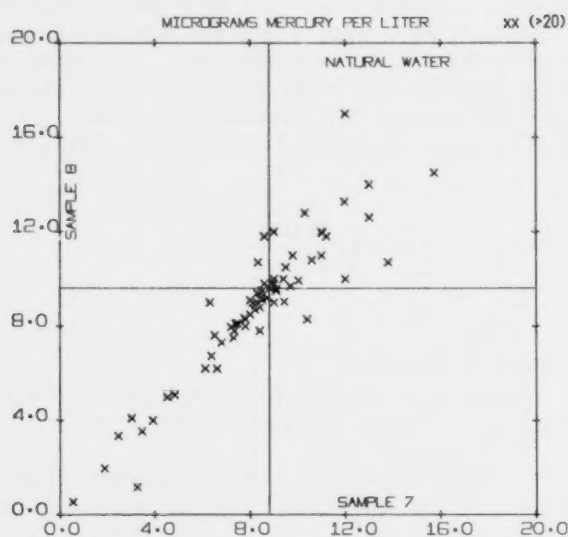


FIG. 8—Two-sample chart for total mercury. Samples 7 and 8.

ever, this did not occur. Systematic error influenced the data plots most, and the distribution of values was not random but on a 45° slope line in the (—) and (++) quadrants, since an analyst obtaining a high or low value on one sample also got a high or low value on the similar sample. This distribution of values on a 45° slope forms an elliptical pattern. Data points far removed from the elliptical cluster indicate a large systematic error for one analyst compared with the other analysts. As random error decreases, data points move closer to the 45° slope. Extreme values or outliers suggest a procedure or instrument out of control. General scatter of data points away from the 45° line indicates poor precision in the method. When all data are spread far out along the 45° line, a large systematic error is indicated for the method. When precision is satisfactory but a significant bias or interference occurs, the data grouping is low (—) or high (++) .

Single Laboratory Precision

In Tables 2 thru 5, the precision measured as standard deviation represents the deviation expected among values received from a number of laboratories. This between-laboratory error is important. It represents the broad error that operates in any mass of data collected from a number of laboratories.

However, another measure of precision important to individual laboratories and analysts is the measurement of how well an individual analyst can expect to perform in his own laboratory. This *within laboratory performance* is measured here as the s_r value. It is used in Table 6 to calculate the single analyst 95% Confidence Interval (95% C.I.) for each set of samples in distilled and natural waters. It was defined by Youden³ as

$$s_r = \sqrt{\frac{\sum (D_i - \bar{D})^2}{2(n-1)}}$$

where

$(n - 1)$ = degrees of freedom,

D_i = difference between values for sample pair, X_i and Y_i , and
 $\bar{D}$ = $\bar{X} - \bar{Y}$ of a series of X and Y values.

The formula measures precision without duplication and hopefully avoids some of the well-intentioned data adjustment that can occur in any laboratory doing replicate analyses.

TABLE 6—Single analyst precision values for mercury analyses.

Sample	Water Type	Level $\mu\text{g/liter}$	95% C.I. ($\bar{X} \pm 1.96s$)
1 and 2	distilled	0.21-0.27	0.42 ± 0.38
	natural	0.21-0.27	0.38 ± 0.32
3 and 4	distilled	0.51-0.60	0.69 ± 0.72
	natural	0.51-0.60	0.71 ± 0.32
5 and 6	distilled	3.4 -4.1	3.77 ± 1.55
	natural	3.4 -4.1	3.51 ± 0.72
7 and 8	distilled	8.8 -9.6	8.92 ± 1.74
	natural	8.8 -9.6	9.01 ± 2.74

Precision Statements

Linear regression analyses were completed on the statistical summary data for mercury analyses in distilled and natural waters. The results of these analyses are shown in Figs. 9 and 10. The precision statements for total mercury analyses in distilled and natural waters in the 0.2 to 10 $\mu\text{g/liter}$ were calculated as follows: in distilled water, standard deviation = $0.1828 + 0.3071 \bar{X}$ (mean); and in natural water, standard deviation = $0.1071 + 0.3855 \bar{X}$ (mean).

The precision of this method for total mercury in distilled water samples within the range of concentration of 0.2 - 10 ug/liter may be expressed as follows:

$$s = 0.1828 + 0.3071\bar{X}$$

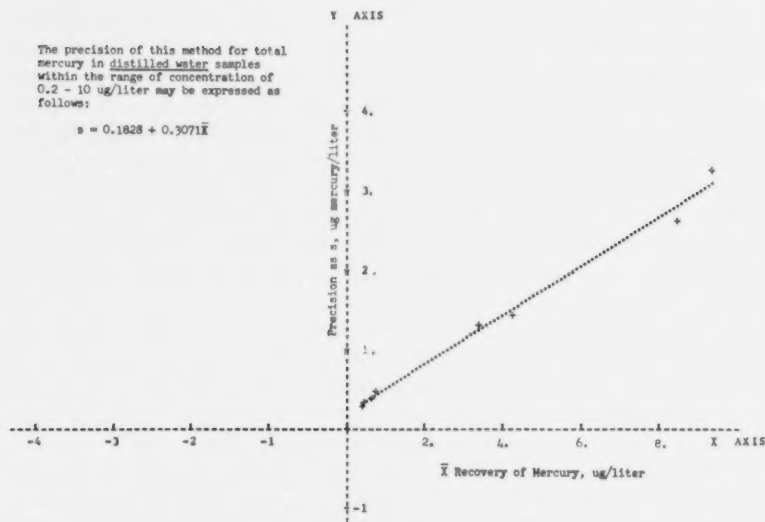


FIG. 9—Precision statement for analysis of total mercury in distilled water.

The precision of this method for total mercury in natural water samples within the range of concentration of 0.2 - 10 ug/liter may be expressed as follows:

$$s = 0.1071 + 0.3855\bar{X}$$

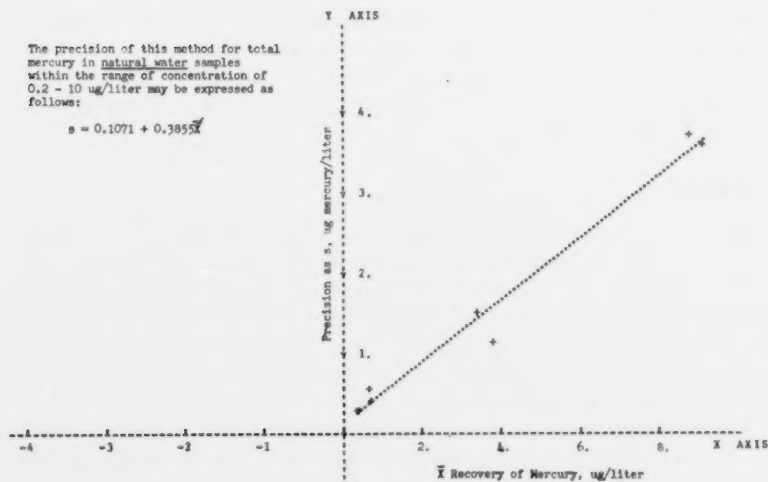


FIG. 10—Precision statement for analysis of total mercury in natural water.

Discussion

The low levels of 0.21 to 0.27 $\mu\text{g/liter}$ total mercury tested here approach the minimum detectable level. The calculated 53 to 98 percent positive bias and a 67 to 79 percent relative deviation are shown graphically in Figs. 1 and 2. Since the measured level is such a small increase above background, any variation in measurement of peak heights will produce fairly large deviations. It is also notable that the water type (distilled versus natural) had relatively little effect on the method. The positive bias is expected because the analyst reporting 0.2 μg as a minimum would report 0, 0.2, or >0.2 values. Hence, positive measurements are automatically 0.2 or more and just as automatically produce a positive bias in the method.

At 0.51 to 0.60 $\mu\text{g/liter}$ levels of total mercury, somewhat above the minimum detectable level, the positive bias in the method is reduced to 18 to 32 percent. This improved bias is evident in Figs. 3 and 4. However, the relative deviation of the data is still at 55 to 80 percent. As with Ampuls 1 and 2, the water type had no particular effect on the results.

At the 3.4 to 4.1 $\mu\text{g/liter}$ level of total mercury, the bias of the method is sharply reduced and is variable, ranging from -7 to $+3.9$ percent. Likewise at this level, the relative deviation is reduced, ranging from 29 to 44 percent. Water type produced no apparent effect on the data. The improved precision and bias, which are obvious in Figs. 5 and 6, sharply contrast the data distribution in Figs. 1 to 4.

At the 8 to 10 $\mu\text{g/liter}$ levels of total mercury, the bias of the method is small and negative, 0.4 to 5 percent, with a relative deviation of 31 to 42 percent. Figures 7 and 8 show data distributions similar to those at the 3 to 4 $\mu\text{g/liter}$ levels, but with a somewhat greater deviation in natural water than in distilled water.

Conclusions

An interlaboratory study was conducted with 90 analysts in 76 laboratories. A series of eight samples in pairs at similar yet different concentrations in the 0.2 to 10 $\mu\text{g/liter}$ range were added to distilled water and natural water of choice by each laboratory. Samples were analyzed using the EPA-ASTM method for total mercury. Precision and accuracy statements, two sample data charts, and single laboratory precision measures were determined on each sample for distilled and natural waters.

The most significant factor in analyses in the 0.2 to 10 $\mu\text{g/liter}$ range is the concentration of mercury itself. At the lowest levels of 0.2 $\mu\text{g/liter}$ which is also taken as the detection limit, the data show a large positive bias of 53 to 99 percent. Similarly, the relative deviations range from 66 to 78 percent of the mean values reported.

As expected, accuracy and precision improve and remain improved as

concentrations increase. Further, as the level of mercury increases, we begin to see natural water samples having a slightly greater standard deviation.

The single analyst precision measures (s_r) are expected to be somewhat smaller than the standard deviations generated for the total data mass. In this study, the s_r values were smaller, relative to concentrations, with s_r values about one half of the total standard deviation at $<1 \mu\text{g/liter}$ levels of mercury. As concentrations increased to mercury levels of 3 to 9 $\mu\text{g/liter}$, the s_r values improved—one half to one fourth of the total precision both in natural and distilled waters.

The precision of the ASTM/EPA method of analysis for total mercury in natural waters is linear within the range tested as follows

$$\text{standard deviation} = 0.1071 + 0.3855 \bar{X} (\text{mean})$$

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